Toxicogenomics Research Consortium Sails into Uncharted Waters

In the current state of gene expression technology, there are various methodologies for assessing gene expression, making it difficult to compare and compile data across laboratories and investigators. To stretch research dollars and coordinate research efforts among scientists who are using genomics to better understand environmental effects on human health, the NIEHS Division of Extramural Research and Training last year funded a $37 million partnership called the Toxicogenomics Research Consortium (TRC). The idea is simple, yet powerful: the synchronization of research efforts of many scientists in various locations promises to produce high-quality results faster and more reliably than isolated research teams working alone.

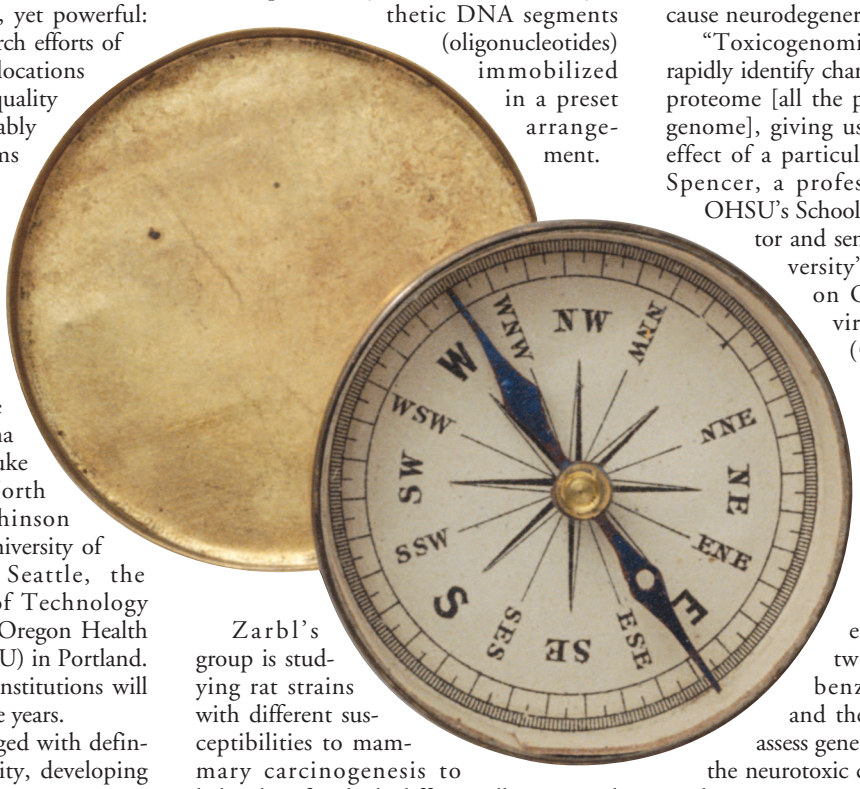
This consortium merges the efforts of investigators with similar environmental health research goals from the NIEHS Microarray Center and five of the country's leading academic research institutions: the University of North Carolina (UNC) at Chapel Hill, Duke University in Durham, North Carolina, the Fred Hutchinson Cancer Research Center/University of Washington (UW) in Seattle, the Massachusetts Institute of Technology (MIT) in Cambridge, and Oregon Health & Science University (OHSU) in Portland. Each of the five academic institutions will receive $7.5 million over five years.

The consortium is charged with defining sites of genetic variability, developing standards for gene expression experiments, and applying gene expression technology to study environmental stress responses in biological systems. These efforts are critical for defining how environmental agents cause disease, identifying biomarkers of disease, predicting chemical toxicity, and understanding why individuals show such a wide variability in their sensitivity to environmental toxicants.

Consortium research projects fall within the realm of environmental toxicogenomics. This emerging discipline of toxicology enables scientists to identify and characterize the genomic signatures of environmental toxicants, as well as use gene and protein expression profiles to study the relationship between exposure and disease and understand gene–environment interactions and their impact on human health.

Research among the Academic Partners

At Fred Hutchinson/UW, principal investigator Helmut Zarbl is leading efforts using DNA microarrays to determine whether particular genes are sensitive to the actions of chemical toxicants and the role of these genes in cancer development. Microarray technology enables researchers to measure the transcription of multitudes of genes simultaneously on tiny microchips containing thousands of targets of complementary DNA (cDNA) or synthetic DNA segments (oligonucleotides) immobilized in a preset arrangement.



Zarbl's group is studying rat strains with different susceptibilities to mammary carcinogenesis to help identify which differentially expressed genes are related to the mechanims of sensitivity. "The ultimate goal is to predict an individual's risk of cancer based on [his or her] genetic profile and environmental exposures," says Zarbl, a member of the center's Human Biology and Public Health Sciences Divisions and an affiliate associate professor of pathology, environmental health, and toxicology at UW.

In other studies at Fred Hutchinson/UW, researchers hope to better understand how environmental factors—metal exposure, nutritional changes, and physical factors such as hyperthermia due to maternal fever—can damage the developing nervous system, and how exposures to certain organophosphates used in pesticides can affect child behavior. Other projects seek to develop tests to measure toxic exposure and stress responses using lab-cultured liver cells.

Researchers at OHSU are collaborating with the Boston Biomedical Research Institute to compare gene and protein expression patterns of nervous system cells in both normal and toxicant-exposed states. Under the direction of OHSU principal investigator Peter Spencer, they hope to increase understanding of the mechanisms underlying toxic diseases of nerve cells and their axons, as well as clarify ways to screen for environmental agents and hazards that have the potential to cause neurodegeneration.

"Toxicogenomics should allow us to rapidly identify changes in the genome and proteome [all the proteins expressed by a genome], giving us a 'fingerprint' of the effect of a particular chemical," explains Spencer, a professor of neurology in OHSU's School of Medicine and director and senior scientist at the university's Center for Research on Occupational and Environmental Toxicology (CROET). That fingerprint, or gene expression profile, he says, allows the team to see if classes of chemicals are working to produce a particular toxicological effect.

CROET researchers have exposed mice to two isomers of diacetylbenzene, one neurotoxic and the other not, hoping to assess gene modulation and isolate the neurotoxic component. By looking at the common gene expression signatures expressed by both agents, they can subtract a portion of each signature, leaving only the shared portion, which reflects changes caused by the neurotoxic element. This element will be compared with the neurotoxic signature of homologous neurotoxic chemicals to determine whether commonalities can be identified.

A second OHSU project will study the effects of a potent genotoxin present in the seed of the cycad, a tropical palm-like plant. Cycad seeds have been used for food and medicines on three Pacific islands—Guam, Papua, and the Kii peninsula of Honshu Island, Japan—where disproportionately high numbers of people have suffered from lytigo-bodig, a rare condition that shares symptoms with amyotrophic lateral sclerosis (Lou Gehrig disease) and Parkinson and Alzheimer diseases. "Understanding the

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cause and pathogenesis of this prototypical neurodegenerative disease may shed light on look-alike disorders worldwide," says Spencer.

On the East Coast, Leona Samson heads up a team of scientists at MIT who are studying the effects of aflatoxin B_1 (which is secreted by mold that grows on grains and is associated with liver cancer) as well as environmental alkylating agents in various cell types, including liver cells. "We're using model systems—animals or cells we know have [well-defined] biological outcomes when exposed to environmental agents—to determine what we can say about the toxicogenomic response," Samson says. "These agents represent toxic agents found in our external environment, our food supply, the endogeneous cellular environment, and the cancer chemotherapy clinic."

Another MIT study involves engineering liver cells to create liver "bioreactors" that process toxic compounds. These three-dimensional structures secrete the protein albumin and even reproduce the force and sheer caused by blood flow, allowing them to mimic how the liver actually functions in a living body. Using the engineered version, scientists will be able to explore how the human liver might respond to toxic insult.

At UNC, researchers from the Lineberger Comprehensive Cancer Center and the Center for Environmental Health and Susceptibility will study profiles of genetic susceptibility to toxicant stress. Led by William K. Kaufmann, a professor of pathology and laboratory medicine in the School of Medicine, UNC researchers are using microarrays to study known environmental and clinical carcinogens as well as nongenotoxic carcinogens, which appear to induce cancer

Christopher G. Reuther/EHP

through mechanisms other than DNA damage. "A protein inside the cells interacts with the compound through specific receptors, which accounts for some or all of its toxicity," explains Kaufmann.

In other UNC studies, researchers will compare gene expression changes in mammary epithelial cells after treatment with various breast cancer therapies, focusing on what appear to be overlapping responses. They will evaluate how genetically pure mouse strains differ in their response to alkylating agents, watching for clues about the genetic predisposition within families to developing a particular cancer.

Researchers from Duke University's School of Medicine and the Nicholas School of the Environment and Earth Sciences, led by David A. Schwartz, a professor of pulmonary and critical care medicine, will watch how novel genes influence an organism's ability to defend itself against endotoxins, cell wall components of gram-negative bacteria. "So far, we've found that there are several important genes expressed that appear to genetically regulate the response to endotoxin," says Schwartz.

A second group of researchers at Duke is studying gene expression in developing zebrafish to see how certain components of retinoic acid synthesis and breakdown pathways are expressed in the neural tube. In a human component of this research, investigators have been studying how variants of the associated genes relate to neural tube defects in children, with results so far ruling out the retinoic acid pathway.

A third project, still in development, will bring together researchers from Duke, Fred Hutchinson, and MIT to study gene response to toxic metals in four different model systems: human cells, mice, zebrafish, and *Caenorhabditis elegans*, a unique roundworm whose genome is fully mapped. "The goal is to identify genes relevant across model systems that are common in all model systems," says Schwartz. "If exposure to toxic agents moderates a gene response in an evolutionarily conserved set of genes or stimulates common gene pathways in multiple species, then [that] narrows the biology of the response," he hypothesizes. "I've been encouraged by the interest among [TRC members] in working together to achieve common goals," he adds. "The cross-species comparisons are only possible with the cooperation of multiple scientists at different locations."

No matter what they are studying, all of the scientists participating in the consortium must wrestle with the "huge question of reproducibility," Samson cautions. "We must compare our results and see where the variabilities come from," she says. "Do they come from certain genes or certain array platforms?" She adds that member scientists should develop initial standards of procedure from the outset "to make sure we're all producing high-quality data."

Research at the NIEHS Microarray Center

Besides the university constituents, the NIEHS Microarray Center participates in the TRC as well. Center director Richard Paules says the NIEHS group is studying the mechanisms involved in nongenotoxic carcinogens, the role that estrogenic compounds play in genetic damage, and human susceptibility syndromes and how oxidative damage contributes to cancer development. In the latter study, Paules says, "we'll focus less on tissue and more on mechanistic pathways to find out the role of oxidative stress in the cancer process. People with inherited susceptibility to cancer lack the *p53* gene that generates cell-cycle checkpoint arrest in DNA. These gene mutations are involved in the DNA damage response." He hopes the group's findings will lead to better diagnoses and treatment therapies for cancer patients.

Paules is currently leading a study of how rodent toxicants affect the gene response in rat livers, kidneys, and peripheral lymphocytes in the blood. "We want to know which exposures are related to disease process effects, not just pharmacological effects," he explains. His group will also search for phenotypic anchors of gene expression changes: "We want to make sure we have a very clear definition of the biology of what's going on in a particular organism, by defining traditional toxicological parameters and making sure gene expression changes are linked to phenotypic changes in the liver models."

Paules looks forward to joining forces with like-minded counterparts at the other TRC member institutions. "Our goal is to be very interactive and collaborate not only with academic partners, but industry as well," he says. He believes this goal will be facilitated by the Chemical Effects in Biological Systems "knowledge base" being developed by the NCT together with the consortium [see "Toxicogenomics: An Emerging Discipline," p. A750 this issue]. "It will contain as much biological, chemical, and toxicological information as possible related to gene expression changes," he explains. "Our goal is to build a publicly accessible database so we can do predictive toxicology." **–Jennifer Medlin**

Two Committees Tackle Toxicogenomics

The advent of toxicogenomics—an emerging scientific discipline that applies recent advances in genomics to the field of toxicology—promises to transform the way scientists use genetic information to study the effects of the environment on human health. Although toxicogenomics may enable researchers to assess the toxicity of environmental pollutants and therapeutic drugs far faster and more economically than traditional methods allow, the field's rapid emergence and fast-paced developments have created a number of challenges as well.

To meet those challenges and navigate a maze of potential technical, regulatory, ethical, and communication roadblocks, the National Research Council (NRC) has formed the Committee on Emerging Issues and Data on Environmental Contaminants. The committee is sponsored by the NIEHS, but because representatives from federal agencies can't participate in NRC committees, a 10-member Federal Liaison Group—composed of representatives from key regulatory agencies such as the Food and Drug Administration, the Environmental Protection Agency, the Occupational Safety and Health Administration, and the National Institute for Occupational Safety and Health—will work alongside the NRC committee.

The NRC committee is composed of 22 experts gathered from academia, private industry, public interest groups, the legal community, and the field of bioethics. Their mission is threefold: to foster open communication among the scientific community and other stakeholders about emerging issues in the field of toxicogenomics, to identify topics for future scientific study, and to consider how data generated by this rapidly evolving technology can best be used to inform public policy and improve public health.

According to David Brown, staff assistant to NIEHS director Kenneth Olden, good communication among stakeholders is absolutely essential. A lack of it can lead to confusion and distrust over the actual implications of environmentally induced health effects, as well as divert attention from the science underlying a particular issue or question. Some data, Brown says, might cause unnecessary public alarm or escape warranted public attention in the hands of uninformed or overzealous members of the

media. Furthermore, media coverage, combined with ensuing public reaction, might unduly affect science policy. An ongoing dialogue among stakeholders will help researchers anticipate problems before they arise and ensure that sound scientific findings guide all decision making. "We thought it would be very important right up front to bring people together to interpret what this data means and communicate it in a responsible way to the public, news media, and other scientists," Brown explains.

Roberta Wedge, program director for risk analysis with the National Academies' Board on Environmental Studies and Toxicology, agrees that proper communication is key. "The [committees] will provide a public forum for communication among different groups on environmental toxicology, risk assessment, exposure assessment, toxicogenomics, proteomics, and bioinformatics," she says. Several times a year—beginning with an inaugural symposium on 4 November 2002—the committees will host open sessions featuring workshops, guest speakers, and interactive panel discussions. Regular newsletters and webcasts are available on the committee's website at **http://dels.nas.edu/emergingissues/**.

The committees' most important discussions will most certainly lead to policy development, Brown believes. "This is a tremendous opportunity for anyone involved in developing regulations using risk assessment," he claims. Marilee Shelton, a program officer with the National Academies' Board on Life Sciences, agrees: "The [committees] will explore how scientific approaches might influence policy and regulatory decisions," she says. Take, for example, changes in gene expression detected with DNA microarrays. Scientists and other parties must not only assess toxicological implications of such changes, but also decide whether those changes will truly result in possibly adverse health effects in humans, she cautions. "Regulatory agencies need to think about what the biological measurements mean."

Other challenges include analyzing enormous quantities of microarray data, standardizing research methods and nomenclature, benchmarking newer toxicogenomic methods against traditional toxicological techniques, developing disease and exposure biomarkers, and extrapolating results from animal experiments to humans—a tricky process at best. As the field of toxicogenomics advances, its ensuing challenges are certain to multiply. These committees stand ready to meet them head-on. **–Jennifer Medlin**

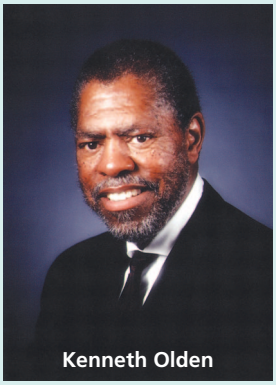
NIEHS

Olden Receives Environmental Public Health Awards

The American Public Health Association (APHA), the world's oldest and largest organization of public health professionals, awarded its prestigious Calver Award to Kenneth Olden, director of the NIEHS and the National Toxicology Program. The award was presented at the association's annual meeting, held 11 November 2002 in Philadelphia.

The award is named for Homer Calver, a World War I medic, public health official, and environmental journalist. Calver was executive director of the APHA and editor of its journal, the *American Journal of Public Health*. He also established and edited the *Environmental News Digest*.

As a health officer in Winston-Salem, North Carolina, Calver brought the city through a diphtheria epidemic and secured a modern ordinance protecting the milk and food supply. Intrigued by European health exhibits he saw on a trip in 1930, he used the APHA to promote American exhibits combining accurate health information with showmanship. One such exhibit, "The Transparent Man," was visited at the 1939 New York World's Fair by 12 million people, a record for a health exhibit that has yet to be broken. The Calver Award was established in 1970, the year Calver died (and the year Earth Day was first observed).


Kenneth Olden

In announcing Olden's award, APHA chair for the environmental section Captain Patrick O. Bohan (Retired) of the U.S. Public Health Service said, "The APHA's environmental section is pleased to recognize the tremendous accomplishments of Kenneth Olden over his more than ten years as director of the NIEHS and NTP, and over his decades in biomedical research. He has deepened the science of his agency at the same time he has broadened its relevance to public health. The recognition is richly deserved."

Since his appointment as director of the NIEHS in 1991, Olden has become a national spokesperson for improved public health through environmental health sciences, with special emphasis on partnerships with grassroots organizations and patient advocacy groups in charting programs of environmental research. He initiated NIEHS-sponsored town meetings throughout the nation that have been public sounding boards focusing on regional environmental health concerns as well as health effects specific to minorities and those of lower socioeconomic status that are caused by exposure to environmental pollutants.

Olden has also taken a leading role in founding the Environmental Genome Project to study individual susceptibility to environmental insults, and the National Center for Toxicogenomics, which applies advances in genetic technology to chemical testing studying environmentally related disease. As recipient of the Calver Award, Olden delivered the keynote address at the APHA's environmental section program at the annual meeting.

In another announcement, Olden has also been awarded the first Cincinnati Children's Environmental Health Award, presented by the Cincinnati Area Lead Advisory Committee (CALAC). The award, presented in November at a reunion of families who have participated in local lead studies, acknowledges Olden's leadership role in addressing children's health issues, especially lead poisoning, and recognizes the role of the NIEHS in a number of children's health initiatives that have enhanced children's health in the Cincinnati area. CALAC is working with the local Community Lead Education and Reduction/AmeriCorps, or CLEARCorps, program to increase community awareness of lead hazards and lobby for passage of a local lead hazard control ordinance.

Fifty Years Later: Clearing the Air over the London Smog

Between 5 December and 9 December 1952, one of the deadliest recorded episodes of urban smog occurred in London, England. New research indicates that as many as 12,000 people may have died as a result of the smog, and mortality from respiratory illnesses such as bronchitis and pneumonia increased more than sevenfold during the smog. Overall death rates during the first half of that month were three times higher than normal, and morbidity and mortality rates in greater London remained elevated well into March of 1953.

The severity of the 1952 London Smog, along with the publicity surrounding it and other smog episodes in the early twentieth century, had two effects. First, they sparked an increased public health effort to understand the effects of air pollution on human health. Second, they prompted the formulation of governmental regulations on air pollution in many countries. This milestone event in the history of environmental health will be commemorated at the conference "The Big Smoke: Fifty Years after the 1952 London Smog," cosponsored by the NIEHS along with the Health Effects Institute, the Wellcome Trust, the Greater London Authority, the London borough of Camden, Sypol (a British environmental health and safety consulting group), the Shell Foundation, and the International Society for Environmental Epidemiology. Organized by Tony Fletcher and Virginia Berridge, professors of environmental epidemiology and history, respectively, at the London School of Hygiene and Tropical Medicine, the conference will be held 9–10 December 2002 at the University of London.

Programs will include historical perspectives of the 1952 London Smog and of air pollution in London in general. London had experienced smog events since the twelfth century, when coal was discovered along England's northeast coast and became the fuel of choice. But such events increased during and after the Industrial Revolution as both manufacturing and the population—both then dependent on burning large amounts of coal—expanded dramatically in the city.

For much of November 1952, temperatures in southern England were unusually low, causing people to heavily stoke their coal-burning home furnaces to keep warm. In the first days of December, high atmospheric pressure over the area caused an inversion that trapped soot and other air pollutants near ground level. Because of the smog, visibility in some areas of central London was reduced to nearly zero for 48 hours. Measurements taken at the time revealed that during that first week of December 370 metric tons of sulfur dioxide were released into the air, where it was converted into sulfuric acid. Large amounts of particulate matter also were released.

Thick as pea soup. A man uses a lit torch to guide a bus through the blinding 1952 London Smog.

Hulton Archive

One seminar at the conference will bring together physicians, researchers, and others who remember the 1952 smog to present their eyewitness accounts of the event. These accounts will eventually be compiled into a book by historians at the London School of Hygiene and Tropical Medicine.

Other presentations will discuss the health impacts of the 1952 smog and the public health response. A presentation by Devra Davis, a visiting professor of public policy at Carnegie Mellon University, will offer a reanalysis of how public health officials handled the event at the time and new evidence indicating that as many as 12,000 deaths may have resulted from the smog. As she wrote in her recently released book *When Smoke Ran Like Water: Tales of Environmental Deception and the Battle against Pollution*, "The true toll of London's smog was hidden for years within official documents. . . . In the 1950s in London and elsewhere, public health statistics were only just starting to be retrievable for use in research."

Even though levels of sulfur dioxide and smoke in London have greatly decreased over the past 50 years, particulate matter remains a significant pollutant, and high levels of other air pollutants such as nitrogen dioxide are being detected, with London residents again experiencing significant winter pollution episodes during 1991, 1994, and 1997. David Hutchinson, an adviser with the Greater London Authority (which oversees economic development and public transportation), will present the current governmental strategy for improving air quality in the city. The plan includes a number of large-scale public transportation and energy initiatives. Proposals include increasing the number of privately owned alternative-fuel vehicles and creating low-emission zones in the city that would bar the most-polluting vehicles from those areas.

Another session will focus on the health effects of urban air pollution worldwide and air pollution episodes caused by vegetation fires. One growing urban center, Dublin, Ireland, has learned a great deal about managing air pollution. A study cofunded by the NIEHS, conducted by researchers at the Harvard School of Public Health, and published in the 19 October 2002 issue of *The Lancet*, found that a 1990 ban on coal sales and coal burning in Dublin has resulted in a 70% decrease in concentrations of black smoke (fine suspended particulate matter), a 15.5% reduction in deaths from respiratory causes, and a 10.3% reduction in deaths from cardiovascular causes. Michael Brauer, a professor in the School of Occupational and Environmental Hygiene at the University of British Columbia in Vancouver, believes the spread of urban areas into formerly forested or agricultural regions is a key factor in the rising incidence of air pollution episodes and human health effects from vegetation fires worldwide.

Although 50 years have passed since the Great London Smog, many people alive at the time vividly remember the smog-choked streets and shrieking ambulance sirens from those days. But thanks to the public outcry following the 1952 smog, they have also witnessed a significant improvement in air quality, public health policy, and government regulation in many countries that has greatly reduced the threat of future smog episodes. **–Erin E. Dooley**